

AN ANATOMICAL STUDY OF THE STEM AND LEAF OF THE MENISPERMACEAE OF SOUTHERN AFRICA*

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ABSTRACT

The anatomy of the stem, petiole and leaf blade is discussed and illustrated. A tentative key, based on the leaf anatomy, is provided to distinguish between the three different species of *Tinospora*.

UITTREKSEL

'N ANATOMIESE ONDERSOEK VAN DIE STINGEL EN BLAAR VAN DIE MENISPERMACEAE VAN SUIDELIKE AFRIKA

Die anatomie van die stingel, blaarsteel en blaarskyf word bespreek en geïllustreer. 'n Tentatiewe sleutel, gebaseer op blaaranatomie, word voorsien om tussen die drie *Tinospora*-spesies te onderskei.

INTRODUCTION

The name "Menispermum" refers to the horseshoe-shaped fruit and/or seeds found in most of the species. With the exception of a single xerophytic Southern African species, the rest of the members of the family are tropical and subtropical lianes, shrubs or suffrutices.

The family is not very large. Willis (1973), recognises 65 genera with 350 species and varieties. According to Troupin (1962), approximately 25 genera with 101 species are found in Africa. 7 Genera with 13 species and 2 varieties occur in Southern Africa.

The small number of species assigned to a relatively large number of genera is interesting when it is taken into account that the family is probably very old. According to Diels (1910), fossilised wood of this family is known from the Tertiary of Hungary and fossilised leaves from the Cretaceous of Northern America and Greenland. Chesters (1957), records fossilised fruit in deposits from the Miocene of Central Africa.

Various authors contributed to our present knowledge on the anatomy of this family, e.g. Prantl (1891), Maheu (1902), Krafft (1907), Solereder (1908 a, b), Rudolph (1909), Diels (1910), Metcalfe and Chalk (1965) and Van der Walt,

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Schweickerdt and Van der Schijff (1970). The approaches of these authors differ greatly. Some of these studies are very synoptic and contain little information regarding the Southern African species while others pay more attention to anomalous growth.

From the taxonomic point of view, a shortcoming in most of these studies is that very little attempt was made to relate anatomical characteristics to the taxonomy of this taxon. This is especially the case with taxa below the rank of family.

In a discussion of the significance of anatomical features and their value in relation to taxonomy in general, Davis and Heywood (1973), conclude that anatomical features may have a value as diagnostics or in determining relationship, but that endomorphic criteria are not of equal value in all taxa.

Due to the fact that very little anatomical information is available regarding the Southern African species, an anatomical survey was undertaken. This was done firstly to obtain more information and secondly to determine how anatomical characteristics could be used as an additional aid in the taxonomy of these taxa.

The following species and varieties occur in Southern Africa. Those marked with an asterisk are endemic.

Cocculus hirsutus (L.f.) Diels

Tiliacora funifera Miers

**Tinospora fragosa* (Verdoorn) Verdoorn et Troupin

T. tenera Miers

T. caffra (Miers) Troupin

Albertisia delagoensis (N.E.Br.) Forman

Stephania abyssinica (Dill. et Rich.) Walp. var. *abyssinica*

S. abyssinica (Dill. et Rich.) Walp. var. *tomentella* (Oliv.) Diels

Antizoma angustifolia (Burch.) Miers ex Harv.

**A. miersiana* Harv.

**Cissampelos capensis* L.f.

C. mucronata A. Rich.

C. hirta Klotzsch

C. torulosa E. Mey. ex Harv.

MATERIAL AND METHOD

The material studied was collected from different localities. At least two specimens of each species were studied, but where some variation was found, additional material from other localities was examined.

The discussion is based mainly on cross-sections through the median part of the stem internode, petiole and lamina. In the case of the leaves, the central part with the midrib was studied.

Fresh material was collected, cut into suitable lengths, fixed in F.A.A. and dehydrated in T.B.A. according to the method of Johansen (1940). It was then

embedded in paraffin wax, cut at $8\text{ }\mu\text{m}$ with a rotary microtome, stained with Safranin and Fast Green and mounted in D.P.X.

RESULTS

The description of the different tissues is given in the same order as seen in cross section from the outside to the inside of the stem and from the adaxial to the abaxial side of the petiole and lamina.

1. Stem

The outline in cross-section varies from elliptic to circular (Fig. 1) and is often lobed.

Epidermis

A cuticle is usually present, even in young stems.

The outer tangential cell walls are usually thickened and cutinised, often up to $12\text{ }\mu\text{m}$ thick as in *Cissampelos hirta* (Fig. 2). This feature was also described by Esau (1965), for *Menispermum*.

In *Cissampelos capensis* the outer tangential walls are usually strongly convex, resulting in the formation of papillae.

Anticlinal cell division often occurs in the epidermis where the development of a phellogen is retarded. This was also recorded by Esau (1965), for other members of this family.

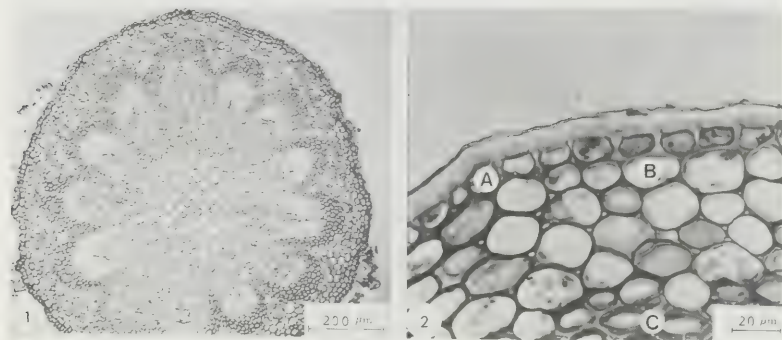


FIG. 1.

Cross-section through the stem of *Cissampelos capensis* illustrating the general internal morphology.

FIG. 2.

Cross-section through the stem of *Cissampelos hirta*. Epidermal cells with cutinised cell walls (A); collenchyma (B) and sclerenchyma (C).

An epidermal phellogen, commonly found in *Albertisia delagoensis* (Fig. 3), was also described for *Tinomiscium petiolare* by Maheu (1902).

Trichomes or hairs usually occur. The most common type is a two-celled uniseriate hair (Fig. 4). Solereder (1908 a, b), Metcalfe and Chalk (1965) and Van der Walt *et al.* (1970), described this same type of hair found in other members of this family.

Multicellular uniseriate hairs as recorded for *Stephania* and *Tinospora* (Metcalfe and Chalk, 1965), were found only in *Stephania abyssinica* var. *tomentella* and not in the three Southern African species of *Tinospora*. None of the above-mentioned authors, however, refer to the multicellular uniseriate weakly-branched hairs (Fig. 5), found exclusively in *Stephania abyssinica* var. *tomentella*.

Stomata of variable size and shape are quite common in the epidermis.

Cortex

This tissue usually occurs as a fairly narrow layer of cells in young stems, e.g. 3–12 cells in *Antizoma angustifolia* or 5–7 cells in *Albertisia delagoensis*. It is clearly differentiated into an outer layer of collenchyma and an inner layer of thin-walled parenchyma cells. Chloroplasts, crystals, tannin and idioblasts (Fig. 6) are often present.

Secretory cells and canals as recorded by Maheu (1906) for *Animirta* are commonly found in *Cissampelos hirta*, *C. mucronata* (Fig. 7), and *Cocculus hirsutus*.

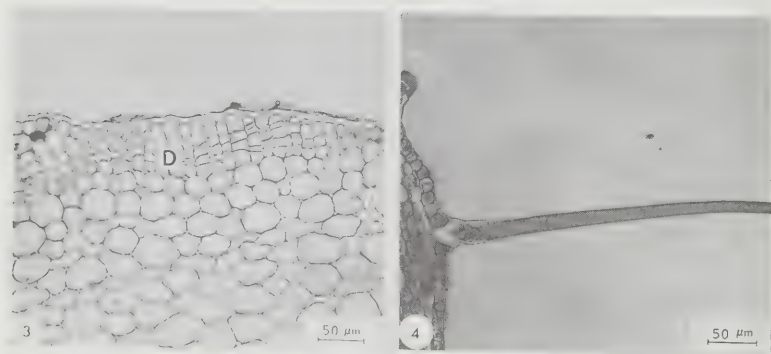


FIG. 3.

Cross-section through the subterranean stem of *Albertisia delagoensis*, showing the epidermal phellogen (D).

FIG. 4.

Two-celled uniseriate hair of *Cissampelos torulosa* as seen in cross-section through the stem.

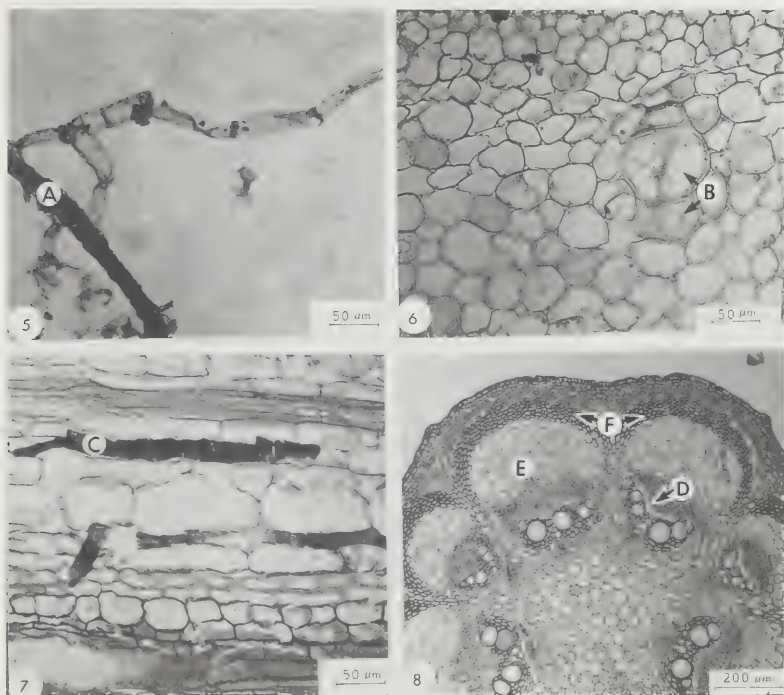


FIG. 5.

Whole mount of multicellular uniseriate branched hairs of *Stephania abyssinica* var. *tomentella*. Part of epidermal cells (A).

FIG. 6.

Cross-section through the subterranean stem of *Albertisia delagoensis*, showing the presence of idioblasts (B) in the cortex.

FIG. 7.

Longitudinal section through the stem of *Cissampelos mucronata*, showing secretory canals with tannin (C).

FIG. 8.

Cross-section through the young stem of *Cissampelos torulosa*. Phloem (D); thin-walled parenchyma tissue (E) and sclerenchyma fibres (F).

The phellogen commonly originates in the cortex of older stems, but in the few exceptions already referred to, may originate in the epidermis.

Pericycle

The term "pericycle" is used here as defined by Metcalfe and Chalk (1965).

In younger stems this tissue is mainly present as a girdle composed of crescent-shaped caps opposite the vascular bundles. It consists mainly of fibres on the outside, and thin-walled parenchymatous tissue to the inside (Fig. 8). In older stems, stone cells usually differentiate opposite the vascular rays between the adjacent caps of fibres (Fig. 9) and the thin-walled parenchyma cells are transformed into fibres (Fig. 24).

Vascular tissue

The bundles are collateral, open and arranged more or less in a circle.

Sieve tubes and companion cells of the phloem are usually easily recognisable and are bordered on the inside by a well-developed vascular cambium. The interfascicular cambium (Fig. 10) is not always easily perceptible in older stems.

The xylem consists mainly of vessels, fibres and parenchyma cells (Figs 11 and 12). Vessels with exceptionally large diameters (up to 150 μm) were found in the stems of the two varieties of *Stephania abyssinica*.

Pith

This tissue is present in all species and generally well-developed. In the semi-succulent stems of *Tinospora* it may represent more than half of the diameter of the stem.

The central pith cells are usually much larger than the peripheral cells and in *T. caffra*, these central cells may have diameters of up to 12 μm .

Starch grains are usually found in the pith of subterranean stems.

In older stems, the medullary rays are composed of fibres.

2. *Petiole*

The outline in cross-section varies from circular with a flattened adaxial side, to oval, reniform, hexagonal or rhomboidal.

Epidermis

A cuticle, although sometimes very thin, is usually present. The characteristics of the outer tangential cell walls and epidermal hairs are similar to those described for the stem.

Cortex

The ratio of cortex width to petiole diameter is much greater than the ratio of cortex width to stem diameter.

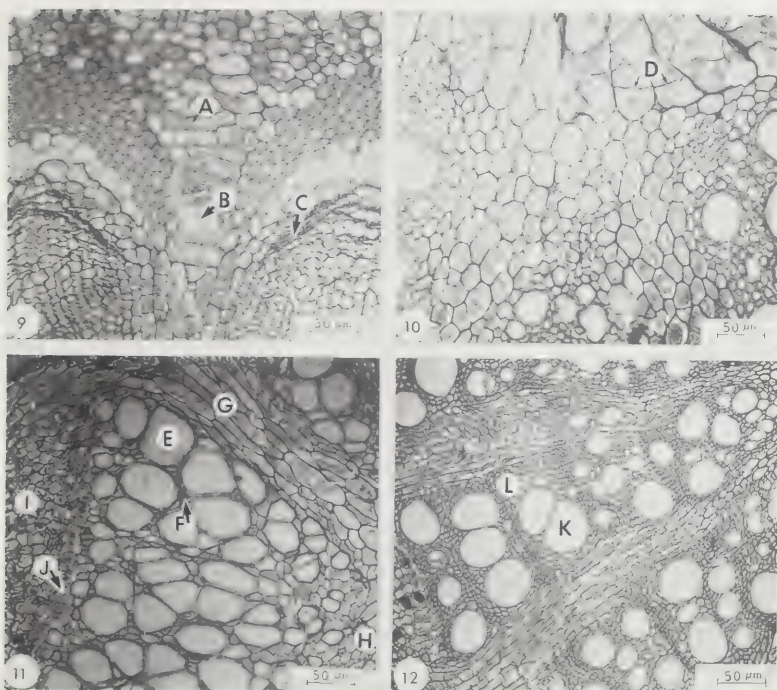


FIG. 9.

Cross-section through the adult stem of *Cissampelos torulosa*. Starch grains (A); stone cells (B) and protophloem (C).

FIG. 10.

Cross-section through the young stem of *Cissampelos torulosa* with interfascicular cambium (D).

FIG. 11.

Cross-section through the vascular bundle of *Stephania abyssinica* var. *abyssinica*. Vessel (E); parenchyma (F); primary vascular rays (G); protoxylem (H); phloem (I) and vascular cambium (J).

FIG. 12.

Cross-section through the secondary xylem of *Cissampelos mucronata*. Vessel (K) and fibres (L).

The cells of the hypodermal collenchyma are usually smaller than the thin-walled parenchyma cells adjacent to the pericycle. The distribution of chloroplasts and tannin is the same as in the stem. No periderm or endodermis were observed in the material examined.

Pericycle

Typical fibre caps, similar to those in the stem, are usually present in *Cocculus hirsutus*, *Tiliacora funifera*, *Tinospora* spp., *Albertisia delagoensis* and a few other species. In *Antizoma angustifolia* and *Cissampelos capensis* the pericycle is represented by sclereids only, while it is completely absent in both varieties of *Stephania abyssinica*.

The thin-walled parenchymatous pericycle usually present in the petioli of younger leaves, is absent in the petioli of young leaves of *Antizoma*. In the older petioli of some of the other genera, it is transformed into fibres.

Vascular tissue

The vascular bundles are similar to those in the stem and are arranged either in the form of a V, e.g. *Antizoma angustifolia* (Fig. 13) or in a circle as in *Cissampelos hirta* (Fig. 14) and the majority of the other species. In both cases, the vascular bundles on the abaxial side were found to be of a larger diameter than the rest.

Pith

This tissue is mainly recognisable in those species with a circular arrangement of vascular bundles. The medullary rays vary from fairly broad e.g. *Tiliacora funifera*, *Tinospora* spp., *Stephania abyssinica* (Fig. 15) to narrow, as in *Alber-tisia delagoensis*.

3. Lamina

Two types of leaves were found, namely isobilateral and dorsiventral. The leaves of *Antizoma miersiana* (Fig. 16) and the narrower leaves of *A. angustifolia* and *Cocculus hirsutus* are isobilateral, while leaves of *Tiliacora funifera* (Fig. 17) as well as the rest of the members of the family, including the broader leaves of *Antizoma angustifolia* and *Cocculus hirsutus* are typically dorsiventral. No centric leaves as described by Krafft (1907), Solereder (1908 a, b) and Metcalfe and Chalk (1965) for other members of the family, were found.

Epidermis

The cells of the adaxial and abaxial surface of the leaves were found to be of more or less the same size, or those of the adaxial surface were somewhat larger.

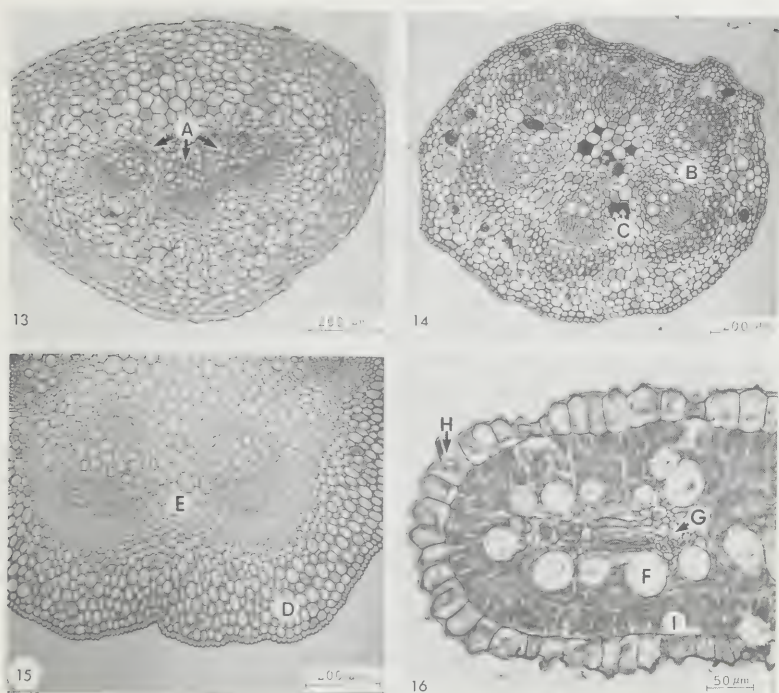


FIG. 13.

Cross-section through the petiole of *Antizoma angustifolia*, showing the outline as well as the vascular bundles (A) arranged in the form of a V.

FIG. 14.

Cross-section through the petiole of *Cissampelos hirta* showing the outline as well as the vascular bundles (B), arranged in a circle. Notice the presence of secretory canals (C) in the petiole.

FIG. 15.

Cross-section through the petiole of *Stephania abyssinica* var. *abyssinica* to show the presence of a well-developed collenchyma (D), the medullary ray (E), and the absence of any sclerenchyma opposite the vascular bundles.

FIG. 16.

Cross-section through the isobilateral leaf of *Antizoma miersiana*. Water tissue (F); Vascular bundle (G); papillose epidermis (H) and palisade parenchyma (I).

The diameter of the epidermal cells of the different species, however, may differ markedly. The largest cells mentioned by Krafft (1908) in a survey of the leaf anatomy of this family, had dimensions of $78 \times 58 \mu\text{m}$. In this regard the epidermal cells of *Tinospora fragosa* (Fig. 18), with dimensions up to $150 \times 150 \mu\text{m}$, are exceptional.

The outer periclinal cell walls of the adaxial surface are cutinised and may be up to $8 \mu\text{m}$ thick as in *Cissampelos hirta*.

Papillose epidermal cells were found in other members of the family by Krafft (1907), Solereder (1908 a, b) and Metcalfe and Chalk (1965). In the Southern African species, this phenomenon is best observed in *Stephania abyssinica* (Fig. 19).

Stomata, usually restricted to the abaxial surface of dorsiventral leaves, are also to be found in the adaxial surface of isobilateral leaves.

No hydathodes as described for *Anamirta* by Krafft (1907), Solereder (1908 a, b) and Metcalfe and Chalk (1965), were found in any of the Southern African species.

Hairs found on both surfaces of the leaves, are similar to those already described for the stem.

An interesting feature in some of the leaves of *Cissampelos torulosa* and *C. hirta*, is the presence of subepidermal mucilage or slime (Fig. 20). As a result of this, the adaxial surface of the leaves exhibits a lead sheen.

Raphides in the epidermal cells of the leaves of *Tinospora fragosa* (Fig. 18) are conspicuous. A survey of other types of crystals of calcium oxalate was given by Solereder (1908 a, b) and Metcalfe and Chalk (1965).

Mesophyll

In isobilateral leaves, e.g. those of *Antizoma miersiana* (Fig. 16), this tissue is not clearly differentiated into palisade and spongy mesophyll.

In dorsiventral leaves, the palisade consists of one cell layer only as in *Tinospora* and *Albertisia delagoensis*. In *Cissampelos*, individual cells of a second layer are often visible, while in *Cocculus hirsutus*, *Tiliacora funifera* and *Stephania abyssinica*, a fully developed second cell layer is often present.

The shape of the cells of the palisade parenchyma may vary from isodiametrical, e.g. *Tinospora fragosa*, to strongly elongated, e.g. five to seven times longer than wide, as in *Tiliacora funifera* and *Tinospora tenera*.

The cells of the spongy mesophyll usually vary in size and shape and are not as closely packed as the cells of the palisade.

In some species, e.g. *Antizoma miersiana*, the parenchymatous or collenchymatous tissue on the adaxial and abaxial sides of the vascular bundles of the midrib is absent so that the mesophyll is continuous (Fig. 21).

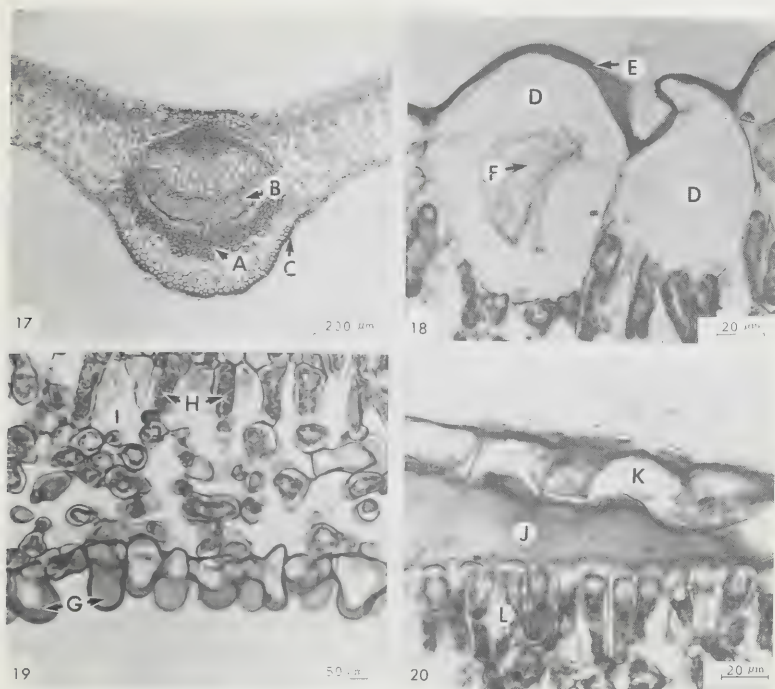


FIG. 17.

Cross-section through the dorsiventral leaf of *Tiliacora funifera*, showing the sheath of sclerenchyma fibres (A), the phloem (B) arranged in the form of a semicircle and the abaxial epidermis (C), usually staining very darkly.

FIG. 18.

Cross-section through the dorsiventral leaf of *Tinospora fragosa* to show the extremely large adaxial epidermal cells (D); the moderately thickened outer cell wall (E) and raphides (F).

FIG. 19.

Cross-section through the dorsiventral leaf of *Stephania abyssinica* var. *abyssinica*. Papillose epidermal cells (G); palisade parenchyma cells (H) and large intercellular spaces (I).

FIG. 20.

Cross-section through the leaf of *Cissampelos hirta* with subepidermal slime (J) between the epidermis (K) and mesophyll (L).

Vascular tissue

Vascular bundles are typically collateral, open and surrounded by a sheath of thin-walled parenchymatous tissue, e.g. *Cissampelos torulosa* (Fig. 22), or sclerenchyma, e.g. *Tiliacora funifera* (Fig. 17). or water tissue, e.g. *Antizoma miersiana* (Fig. 16).

Tannin is commonly found in the cells of the bundle sheath, e.g. *Cissampelos hirta* (Fig. 23).

The hypodermal parenchyma on the adaxial and abaxial sides of the bundle is often replaced by collenchyma, e.g. *C. torulosa* (Fig. 22).

The xylem consists mainly of vessels and parenchyma. A vascular cambium is usually visible in the bundles of older leaves, such as *C. torulosa* (Fig. 22).

Sieve tubes and companion cells of the phloem are not always easy to distinguish. In some species, e.g. in the isobilateral leaves of *Cocculus hirsutus*, or the dorsiventral leaves of *Tiliacora funifera* (Fig. 17), the phloem is arranged in the form of a semi-circle around the xylem.

DISCUSSION

It is significant that, although some adult stems may become semi-succulent, e.g. the different *Tinospora* spp., or woody with diameters up to 180 mm and 220 mm as in *Cocculus hirsutus* and *Tiliacora funifera* respectively, the same basic anatomical structure is found in the younger stems of all species. Even in those that become woody at a very early stage, such as the xerophytic *Antizoma miersiana* this phenomenon is observed. This is quite remarkable when taking the long historical background of this family into account. Adaptation to different environmental conditions thus seems to have little effect on the anatomy of the young stems.

Anatomically the leaves of the different species exhibit greater diversity than the stems. The Southern African species of *Tinospora* can serve as a good example, where anatomical characteristics can be used as an aid to identification where the traditional exomorphological approach of the vegetative parts offers little help in distinguishing between species.

TENTATIVE KEY FOR THE IDENTIFICATION OF THE DIFFERENT *TINOSPORA* SPECIES, BASED ON THE INTERVEIN ANATOMY OF THE LEAF

Adaxial epidermal cells of variable size and shape; ratio of anticlinal width of largest of these cells to leaf thickness $\pm 1:2$; raphides in epidermal cells common **T. fragosa**
 Adaxial epidermal cells of more or less uniform size and shape; ratio of anticlinal width of these cells to leaf thickness $\pm 1:4-1:5$; raphides in epidermal cells not very common.

Cells of palisade parenchyma short; $\pm 1-3$ times longer than wide **T. caffra**

Cells of palisade parenchyma elongated; $\pm 5-7$ times longer than wide **T. tenera**

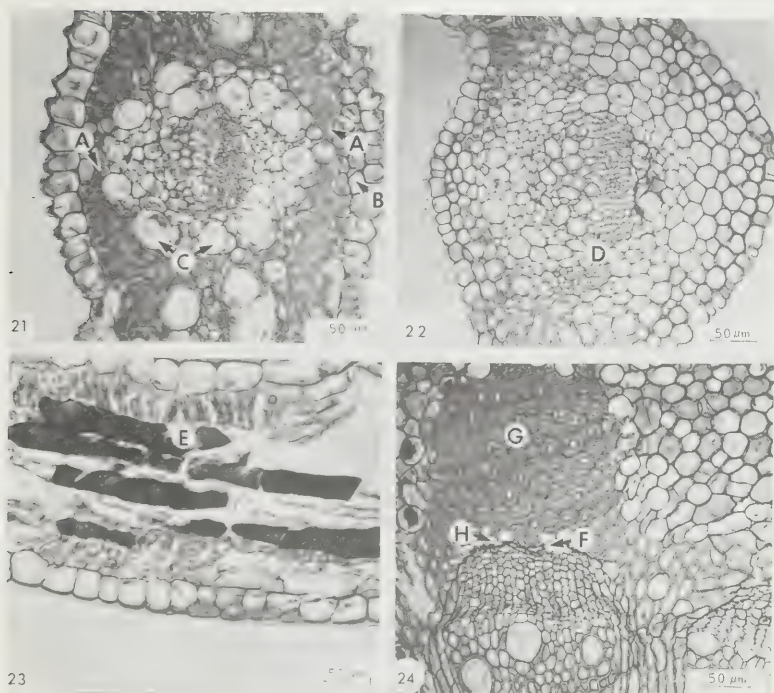


FIG. 21.

Cross-section through the midrib of *Antizoma miersiana* with the continuous mesophyll (A) on the adaxial and abaxial sides of the vascular bundle; collenchyma (B) and water tissue (C).

FIG. 22.

Cross-section through the midrib of *Cissampelos torulosa* to show the vascular cambium (D) and the absence of any fibres in the bundle sheath.

FIG. 23.

Cross-section through the leaf of *Cissampelos hirta* showing longitudinally sectioned tanniniferous bundle sheath cells of a lateral vein.

FIG. 24.

Cross-section through the stem of *Antizoma angustifolia* showing the limited amount of thin-walled parenchyma (H) between the sclerenchyma fibres (G) and protophloem (F).

CONCLUSION

With the exception of suggesting a possible close relationship between the different members of this family, the anatomy of the stem proved to be of little value in delimiting the different species.

Contrary to this, the anatomical characteristics of the leaf can be used as an aid in solving taxonomical problems in the Southern African Menispermaceae. Such anatomical characteristics are:

- (i) The type of epidermal cells.
- (ii) The number of layers of palisade parenchyma.
- (iii) The absence or presence of tannin in certain cells.
- (iv) The characteristics of the sclerenchyma.

Data from these characteristics are incorporated in a numerical taxonomic survey of this group which will be published elsewhere.

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